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Repeat wt-1 peptide binding Assay

5x10⁵ T₂ cells/well in RPMI + 5% Boiled FCS.

Add peptides to a final conc. of 100 nM.

Leave to binding at 37°C 7h

wash x1 with PBS + 2% FCS.

Apply mAb A2 mAb HB54, HB117 30' on ice

wash x3

Apply G & M FITC (1/250) 30' on ice

wash x3

Resuspend cells in PBS (400ul).

FACS Analysis

Results, Good.

peptide

— mAb only

Mean: HB54

513

HB117

HPV E7/86-93

HRV

WT-1 / 10-

WT-1 / 17-

WT1 / 126-

WT1 / 187-

WT1 / 225-

WT1 / 235-

WT1 / 280-

WT1 / 441-

37.70

~~100.20~~

146.86

~~2162~~

~~93.04~~

43.79

69.78

93.01

72.03

95.63

45.66

137.52

42.32

118.12

220.67

144.38

~~54.04~~

~~30.79~~

136.27

97.56

105.90

70.42

172.31

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A2 Binding Titration

2

peptides

WT1

10

17

126

187

225

235

280

441

HPVE7 EG-93

HBV 17-28

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Plate peptide in RPMI + 5% BSA FCS in 96 well plate (U bottomed). from 100 μ M of first column to column 11 (0.0001 μ M) in 50 μ l medium.

Add 50 μ l of T2 cells at a conc of 6×10^6 / ml i.e. 5×10^5 / well mix well.

37°C overnight.

wash x 1 \times PBS (cold) + 1% FCS

stain \times HB 117 and HB 54 (next culture \times) 5 μ l.

for 30' on ice

wash x 3

Apply Ga M Ig FITC at 1/250 (50 μ l).

for 30' on ice

wash x 3

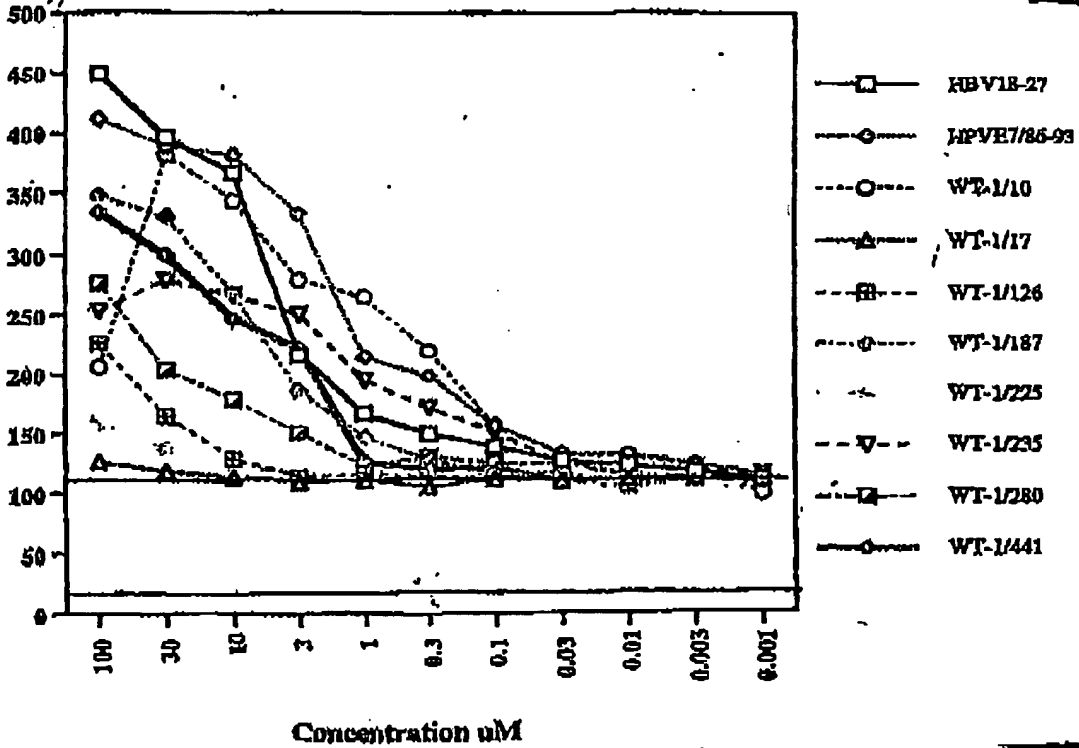
Analyse on FACS

Results: Good. See attached sheets.

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3

WT-1 peptides binding to A2 molecules on T2 cells
--- Stained with HB54 mAb



Binding of WT-1 peptides to A2 molecules on T2 cells
--- Stained with HB117 mAb

